
**UPPER MISSOURI RIVER
ARCTIC GRAYLING
CONSERVATION STRATEGY**



Prepared by
MONTANA ARCTIC GRAYLING WORKGROUP
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Upper Missouri River Arctic Grayling Conservation Plan

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Introduction and Overview

Arctic grayling *Thymallus arcticus* (grayling) were once widespread in the Missouri River Basin upstream of Great Falls, MT (UMR), but indigenous populations are now restricted to the upper Big Hole and Centennial valleys. Conservation populations, which were developed using UMR grayling, have been established in historically occupied habitats including the Ruby River, several tributaries to the Big Hole, and the upper Madison River drainage. Additionally, 12 populations that represent the ancestral genetic variation of UMR grayling have been established in historically unoccupied mountain lakes within the UMR. Grayling are a unique native species and an important part of Montana's history and natural heritage.

UMR grayling are listed as *Species of Concern* by the State of Montana and a *Sensitive Species* by the U.S. Bureau of Land Management (BLM) and the U.S. Forest Service (USFS). Though previously identified as a *Candidate Species* under the Endangered Species Act (ESA), in 2014 the U.S. Fish and Wildlife Service (USFWS) found that listing UMR grayling as threatened or endangered was not warranted. This finding was confirmed in 2020 following legal challenge (USFWS 2020).

Management agencies with an interest in conservation and restoration of UMR grayling formed the Montana Arctic Grayling Workgroup (AGW) in 1987 following statewide status concerns. AGW is chaired by Montana Fish, Wildlife & Parks (FWP), and includes representatives from FWP, Montana Department of Natural Resources (DNRC), Natural Resources Conservation Service (NRCS), USFWS, BLM, USFS and Yellowstone National Park (YNP). AGW developed the *Montana Fluvial Arctic Grayling Restoration Plan* in 1995 that called for establishing at least five fluvial conservation populations among at least three of the subbasins within the historic range. Following creation of the plan, numerous approaches have been used to conserve and restore Arctic grayling in Montana. Conservation actions have improved habitat, conserved genetic diversity, expanded distributions through introductions to suitable habitats, and developed a better understanding of grayling biology, life history requirements, interactions with nonnative trout, and habitat needs. In 2016, a Memorandum of Understanding (MOU) formalized commitment among AGW members to develop an updated conservation plan that addresses all UMR grayling populations and approaches (AGW 2017).

This Conservation Strategy was prepared by AGW to update past conservation plans and describe contemporary conservation approaches and programmatic needs. This strategy incorporates the most promising elements of established conservation approaches, techniques, and research and builds upon the success and knowledge gained since the establishment of the AGW to provide guidance for conservation of UMR grayling. The Strategy updates grayling status and includes objectives, goals, strategies, and measures for all conservation populations. It prioritizes conservation programs in the Big Hole and Centennial valleys, including implementation of Candidate Conservation Agreement with Assurances (CCAA) programs in both watersheds and an adaptive management plan for Centennial Valley grayling. This diverse strategy requires support and collaboration among a large group of partners, which includes the Montana Chapter of the American Fisheries Society, Montana State University, Montana Trout Unlimited (MTU),

The Arctic Grayling Recovery Program (a 501 (c) nonprofit), The Nature Conservancy (TNC), The University of Montana, The University of Montana Western, Big Hole River Foundation, Big Hole Watershed Committee, Northwestern Energy, Centennial Valley Association (CVA) and numerous private landowners who provide access to the habitats where many Arctic grayling conservation and restoration efforts occur. Resource agencies are encouraged to collaborate with partners to meet the intent of this Strategy and direct UMR grayling restoration efforts within their authority. The AGW anticipates that the actions described in this Strategy will be implemented, leading to the long-term conservation and persistence of UMR grayling in Montana. This Strategy incorporates new information to revise the original 1995 Restoration Plan and will be updated hereafter every five years.

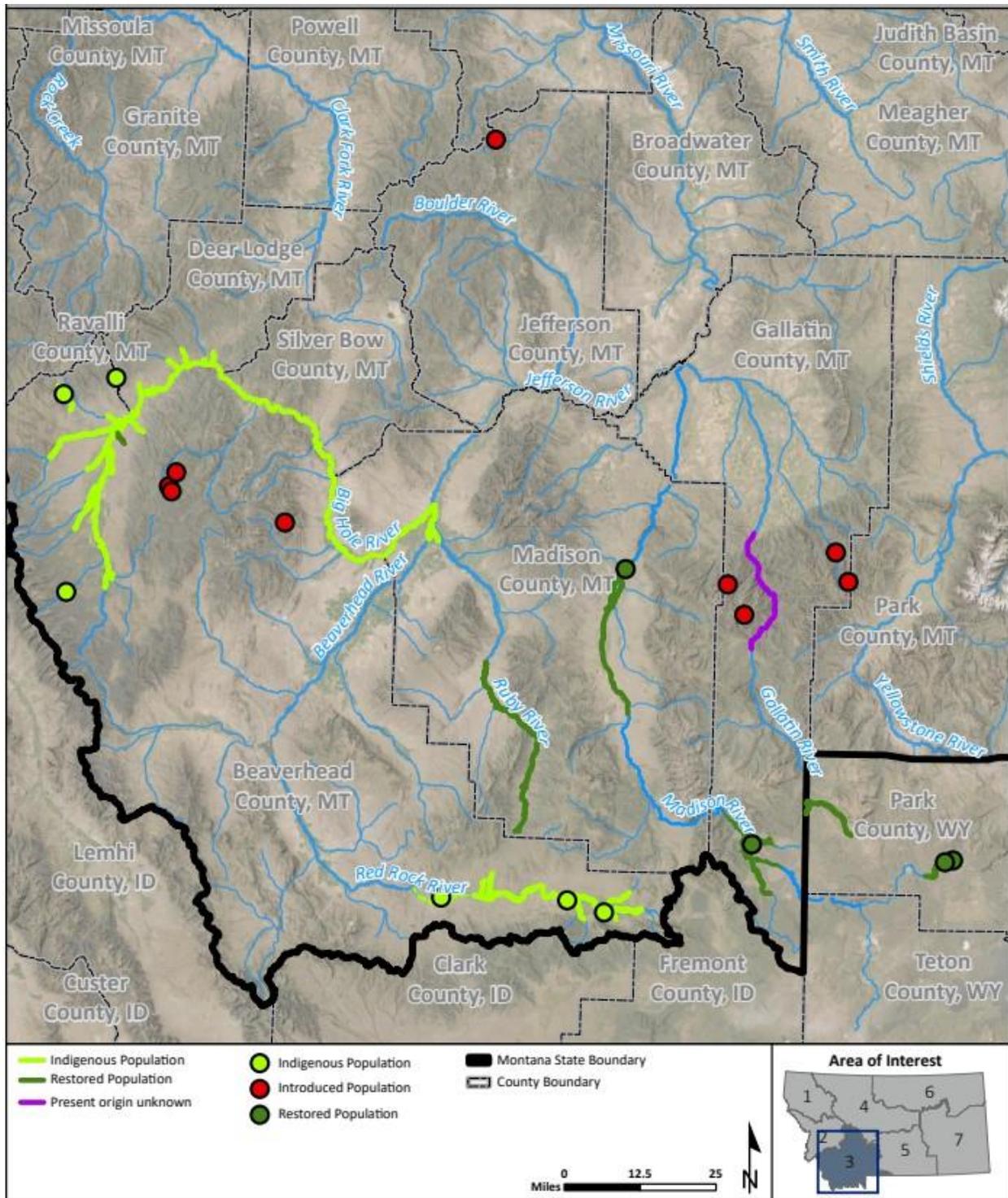


Figure 1. Current distribution of Arctic grayling in the upper Missouri River Basin in streams (lines) and lakes (dots). The distribution of grayling includes extant indigenous populations (bright green), introduced conservation populations (red), reintroduced native populations (dark green), and a population of unknown origin in the Gallatin River (purple). Lake Levale in the Sun River is not shown due to map scale.

Historical and Current Distribution

UMR grayling distribution and abundance has declined over the past 150 years, particularly in riverine habitats (Vincent 1962; Kaya 1992). Grayling were patchily distributed throughout the UMR drainage prior to the mid-1850s and historically inhabited the mainstem Missouri River and the Smith, Sun, Jefferson, Madison, Gallatin, Big Hole, Beaverhead, Red Rock, and Ruby drainages (Vincent 1962; Kaya 1992). Grayling also occupied Red Rock and Elk lakes in the Centennial Valley (Vincent 1962; Kaya 1992) and Miner, Mussigbrod, and Pintler lakes in the upper Big Hole River drainage (Leary et al. 2015). Currently, there are 19 extant UMR grayling populations (Figure 1). Riverine populations of grayling exist in the Big Hole River and its tributaries, Red Rock Creek and its tributaries, the upper Ruby River, and the headwaters of the Gibbon River in the Madison River drainage (Kaya 1992; Byorth 1997; Liermann 2001; Gander et al. 2019, Puchany 2021). Extant lake populations in the UMR reside in Miner, Mussigbrod, and Pintler lakes in the upper Big Hole River drainage (Leary et al. 2015). Twelve additional mountain lake populations in the Gallatin, Madison, Big Hole, Missouri, and Sun rivers drainages are the result of historical stockings and are important for genetic conservation of Arctic grayling in the UMR (Leary et al. 2015; USFWS 2020). Contemporary abundance and genetic status of each population is described below.

Threats

Habitat Degradation: Habitat degradation reduced the distributions of native fishes within the UMR, beginning with European settlement of western Montana in the 1800s (Vincent 1962). Dam construction on the Madison and Red Rock rivers inundated essential spawning habitats and blocked fish movements (Kaya 1990, Vincent 1962). Early agricultural practices contributed to population declines of grayling in several large rivers of Montana through dewatering, degradation of riparian habitats, and siltation (Vincent 1962). Extensive irrigation occurred from most tributaries by the early 1900s and complete dewatering of streams for irrigation, especially during periods of drought, likely had a large influence on distribution, abundance, and life history strategies of grayling through time (Deeds and White 1926, Vincent 1962, Randall 1978). In addition to dewatering streams, irrigation diversions can entrain grayling and restrict their movements (FWP 2021). Overgrazing and the introduction of nonnative grasses in riparian areas led the replacement of deep-rooted native willows, sedges and grasses by shallow-rooted species, which resulted in increased streambank erosion, over-widened channels, decreased pool habitat, and increased water temperatures (Hansen et al. 1995). Extensive cattle grazing in the Centennial Valley resulted in excessive siltation, which contributed to reduced depths in Upper Red Rock Lake (Upper Lake) from over 20 feet to less than six feet (Gangloff 1996). In the Centennial Valley, waterfowl management and construction of instream ponds to benefit the once-endangered trumpeter swan disconnected spawning tributaries, blocked migrations, and inundated spawning gravels (Vincent 1962; Gillin 2001). Improved grazing and irrigation practices and targeted habitat programs and restoration projects have improved instream flows, riparian health, fish passage, and grayling abundances (USFWS 2020).

Nonnative Species: The influence of nonnative fish on grayling varies by species. Nonnative trout have replaced native salmonids, except for mountain whitefish, in much of the UMR (Miller et al. 1989; Nico and Fuller 1999; Schade et al. 2005). Sympatric populations of introduced brook *Salvelinus fontinalis*, rainbow *Oncorhynchus mykiss*, and brown trout *Salmo trutta* may compete with and prey on UMR grayling (Nelson 1954; Miller et al. 1989; Streu 1990; Fausch 1998; Katzman 1998; Schade et al. 2005). Brook trout, which are the most widespread nonnative fish in the UMR, prey on grayling eggs (Katzman 1998). However, in the Big Hole River, there is little evidence that brook trout limit grayling abundance or distribution (Magee and Byorth 1994). Conversely, high brown trout densities were identified as a primary limiting factor for age-0 grayling in the Big Hole River (McCullough 2017). Brown trout were also considered to be a primary factor in the decline and subsequent unsuccessful grayling reintroductions in the Madison River near Ennis (Vincent 1962, Clancey 1997, Liermann 2001, FWP 2014). In the Centennial Valley, nonnative Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri*, were not primary drivers of grayling abundances (Katzman 1998; Warren et al. 2022).

Overharvest: Overharvest and early propagation programs which removed millions of gametes per year from indigenous populations contributed to historical grayling declines. Grayling are generally more susceptible to angling than nonnative trout, and liberal historical creel limits enabled overharvest of grayling (Henshall 1907; Beal 1953). High fishing pressure in the early 1900s caused declines in grayling abundances in parts of the UMR, which was exacerbated when overall angling pressure increased throughout Montana following the establishment of nonnative trout fisheries (Vincent 1962). Additionally, initial propagation programs removed millions of gametes per year from the Centennial and Madison River grayling populations as other threats emerged in the UMR (FWP 1907, Beal 1953). Protective regulations (e.g., catch-and-release for grayling on all rivers, spawning closures in Red Rock Creek) and increased enforcement have reduced this threat (FWP 2014). However, high fishing pressure, particularly during warm summer months, may still result in incidental grayling mortality.

Climate Change: Measured and predicted changes in the mean or variability of one or more measures of climate (e.g., temperature or precipitation) were defined by the USFWS as a potential threat to UMR grayling persistence (USFWS 2020). Freshwater ecosystems are generally the most sensitive to deviations in global temperature and resulting shifts in habitat conditions (Comte et al. 2013). Glacial-relict species such as UMR grayling, may be vulnerable to environmental changes because they evolved in Montana under much cooler conditions and currently exist in peripheral populations which are generally near their limits of ecological tolerance (Vincent 1962). Systemic habitat improvement and targeted intervention will improve grayling resiliency to climate change. Climate change has reduced annual precipitation and snowpack levels, diminished the magnitude of spring runoff, and increased water temperatures in Montana (Lohr et al. 1996; Gillilan and Boyd 2009; Vatland 2015). A warming climate could have negative consequences for grayling through increasing water temperatures and exacerbation of impacts from agricultural practices (Vincent 1962). Decreased precipitation and snowpack levels in the Centennial Valley have led to lower water depths and increased retention times in Upper Lake, which has contributed to reduced amount of suitable overwinter habitat for grayling (Davis 2016; Warren et al. 2022). Elk Lake contained a viable population of grayling until

the 1990s when significantly lower annual precipitation and geologic activity disrupted connectivity to spawning tributaries (Lund 1974; Gillilan and Boyd 2009). Targeted intervention and unconventional projects may be required to address these threats (e.g., Flynn et al. 2019). In the Big Hole River, ongoing drought combined with early runoff has decreased mean summer discharges (Vatland 2015); however, the Big Hole CCAA has improved irrigation practices, stream flows, and riparian habitats, which provide a critical buffer against climate change (FWP unpublished data; Seavey et al. 2009).

Conservation Objective, Goals, Strategies and Measures

Conservation Objective: Ensure the long-term, self-sustaining persistence of UMR grayling.

Conservation Goals: The conservation objective will be achieved when the following goals are met:

1. The indigenous Big Hole River, Centennial Valley, Miner Lake, Mussigbrod Lake, Pintler Lake and viable introduced populations (defined below) exhibit a stable or increasing genetic effective population size (N_e) over multiple generations that is sufficiently large to avoid inbreeding depression and maintain evolutionary potential.
2. The geographic distribution of extant UMR grayling is maintained or increased.
3. Self-sustaining conservation populations of UMR grayling (defined below) are restored in historically occupied drainages.

Conservation Strategy: This Strategy emphasizes the conservation of genetic variation in UMR grayling, and thus, the evolutionary legacy of the species. The strategies to achieve the conservation goals for UMR grayling are specific to each drainage because the issues and complexities of grayling conservation are unique across the landscape. Drainage-specific strategies involve managing for local population sizes sufficiently large to avoid loss of genetic variation and maintain evolutionary potential (Goal 1), and in the event of local population decline or extirpation, replication of genetic variation across multiple, independent populations throughout the UMR (Goals 2 and 3). Drainage or population-specific strategies to meet each conservation goal and, resultantly, the overarching conservation objective are described in detail below. In most cases, the specified conservation strategies are designed to simultaneously meet multiple goals. Additionally, population expansion opportunities for UMR grayling into suitable riverine and lake habitat will be considered in conjunction with all fish removal projects intended to expand the distribution of westslope cutthroat trout *Oncorhynchus clarkii lewisi* (WCT) (e.g., French Creek, Selway Creek). The WCT Conservation Strategy for the Missouri River Headwaters of Southwest Montana (FWP 2022b) outlines potential projects to restore WCT over large reaches of tributary streams not presently occupied by grayling.

Measures of Success and Monitoring: Attainment of conservation goals for each population will be objectively determined by genetic (Goal 1) and presence/absence (Goals 2 and 3) monitoring.

Goal 1: Status of indigenous and viable introduced UMR grayling populations will be assessed by directly estimating or considering genetic metrics relevant to N_e . A genetically viable

conservation population of UMR grayling is defined as one in which N_e is 1) sufficient to maintain adaptive genetic variation; 2) sufficient to minimize risk of inbreeding depression; and 3) either stable or increasing over time. N_e controls the rate at which populations lose genetic variation (i.e., evolutionary potential) due to genetic drift and the rate at which inbreeding accumulates in small populations. N_e is a critical measure in conservation biology (Allendorf et al. 2022), but one of the most difficult metrics to accurately estimate in wild populations (e.g., Waples et al. 2014). Accurate estimates of N_e require extensive sampling that is not realistic for most extant UMR grayling populations. As such, N_e will only be monitored explicitly for the Big Hole and Centennial Valley populations because they 1) have the highest priority conservation status in the UMR, 2) are most strongly limited by human actions or the legacy of human actions, and 3) are the specific targets of large-scale conservation programs where annual estimates of population success are critical for communicating the efficacy of conservation actions with stakeholders. The effective population size will be estimated using annual estimates of the effective number of breeders (N_b), the number that produce a given cohort and multiple life history traits (Waples et al. 2013; Waples et al. 2014) for the Big Hole and Centennial Valley populations (Table 1). In the Big Hole drainage, N_b estimates will be produced by collecting samples from at least 120 young-of-year (YOY) grayling from known spawning locations across the drainage each fall. In the Centennial Valley, N_b will be estimated by sampling spawning fish from Red Rocks and Elk Springs creeks and assigning cohorts based on age determined from scales. For all other indigenous and introduced UMR grayling populations, inference about N_e will be based on temporal trends of allelic richness (A_r) and average heterozygosity (H_e) every six to eight years (Tables 1 and 2). Allelic richness is the number of alleles present in a population, which is a surrogate for standing genetic variation. Average heterozygosity is a fundamental measure of genetic variation and represents the proportion of genes that have two different alleles within a population. Allelic richness provides information about adaptive potential (e.g., Caballero and García-Dorado 2013; Allendorf et al. 2014), whereas H_e assesses the risk of contemporary and future inbreeding depression. Both measures are directly influenced by N_e , where populations with increasingly small N_e experience increasingly rapid loss of H_e and A_r , with A_r being particularly sensitive to rapid declines in N_e (Allendorf 1986; Luikart et al. 1998). However, neither metric increases with increasing N_e over contemporary management time-scales. Broadly speaking, temporal stability in measures of genetic variation over a few generations provides evidence that N_e is relatively large, whereas decreases provide evidence that N_e is small. Thus, losses of genetic variation can be used to trigger management actions that can help ensure conservation goals are achieved.

Goal 2: Distribution of extant UMR grayling populations will be monitored with electrofishing, eDNA, or netting. Frequency of distributional sampling for UMR grayling populations will occur on a case-by-case basis as dictated by genetic trends.

Goal 3: Introduced grayling will be designated as self-sustaining UMR conservation populations when they are located within the UMR, founded using UMR grayling, and persist for 10 years with no artificial propagation and wild fish from each cohort are observed. Reintroduction approaches will include demographic and genetic criteria. Each introduced population will be established using source populations selected to maximize their conservation value and genetic diversity and founded by at least 50 breeding pairs to reduce genetic bottlenecks. Successful

establishment of non-indigenous lake populations in Montana typically involved stocking 1,500-2,000 grayling per acre for at least a three-year period. Based on results from the Ruby River reintroduction, onsite incubation of fertilized eggs will be the primary introduction tool for fluvial populations. All stream reintroductions should utilize at least 130,000 eggs per year for five years with at least two years exceeding 200,000 eggs (Gander et al. 2019). In lakes, a combination of stocking fry, fingerlings, and onsite incubation will be used. Monitoring to assess whether a population is self-sustaining will begin after stocking ceases and natural reproduction of introduced fish is expected. When an introduced population meets criteria to be designated as a self-sustaining UMR conservation population its genetic status (A_r , H_e) will be assessed as described above.

Table 1. Genetic sampling intervals for estimates of the number of effective breeders (N_b), effective population size (N_e), allelic richness (A_r), and expected heterozygosity (H_e) for indigenous Arctic grayling populations in the upper Missouri River Basin.

Population	Genetic Metric	Sampling Interval
Big Hole	N_b , N_e , A_r , H_e	Annual
Centennial Valley	N_b , N_e , A_r , H_e	Annual
Miner Lake	A_r , H_e	6-8 years
Mussigbrod Lake	A_r , H_e	6-8 years
Pintler Lake	A_r , H_e	6-8 years

Table 2. Self-sustaining, introduced Arctic grayling populations in the upper Missouri River Basin, by subbasin. These populations will be monitored for H_e and A_r every 6-8 years.

Subbasin	Populations
Big Hole	Agnes, Bobcat, Schweinegar, Odell
Beaverhead	Ruby
Madison	Gibbon
Gallatin	Hyalite, Deer, Emerald, Grayling
Sun	Levale, Gibson, Diversion,
Missouri	Park

Drainage-specific Conservation Strategies

Conservation strategies for the next five years have been developed for each of the 19 extant UMR grayling populations. These strategies describe all measures that are planned for each population to achieve the overarching conservation goals and objective for UMR grayling. The operations cost to implement the UMR conservation strategy over the next five years is about \$2.7 million and will require the time of at least 34 staff. Costs to implement conservation measures include on-the-ground projects, contracted services, general operations, and genetic analyses. Staff refers to the total number of personnel that are required to implement each conservation measure, with the time spent by each of the specified staff varying from several weeks to an entire year and multiple conservation measures being implemented by the same

personnel each year over the duration of this strategy. The requisite staffing to execute all conservation strategies is presently provided by FWP, DNRC, USFWS, NRCS, USFS, BLM, YNP, CVA, MTU, and TNC.

Big Hole River: Indigenous fluvial Arctic grayling inhabit the mainstem of the Big Hole River, downstream reaches of at least 11 tributaries, and seasonally connected side channels (Figure 2). The Big Hole River is a moderate-sized, free-flowing river system that originates in Skinner Meadows near Jackson and flows about 150 miles to its confluence with the Beaverhead River to form the Jefferson River near Twin Bridges. The upper Big Hole Valley is wide and flat, fed by dozens of tributaries, and supports numerous cattle ranches dependent on irrigated hay and pasture. The Big Hole River grayling population is the last indigenous, entirely fluvial grayling population in the lower 48 states (Shepard and Oswald 1989) and has been the center of focused research (Lohr et al. 1996, Byorth and Magee 1998, Vatland 2015, McCullough 2017); management, and conservation (Kaya 1990, Kovach et al. 2019, USFWS 2020, FWP 2021) since the 1970s.

The distribution and abundance of Big Hole grayling populations declined to historic lows by the late 1990s but have since improved following targeted conservation. Consistent monitoring of the Big Hole River grayling population began in the 1970s (Liknes 1981) and declines were observed at most monitoring sites by the late 1980s (Shepard and Oswald 1989). Declines were likely triggered by low-flow conditions that resulted in weak cohorts of grayling (Shepard and Oswald 1988; Kaya 1990). Historically, irrigation and stock water withdrawals completely dewatered the Big Hole River during drought periods. In 1988 the Big Hole River near Wisdom was completely de-watered for 24 days. Age-1 and older grayling abundances in the Wisdom Section decreased from 69/km in 1983 to 14/km in 1989 and the population continued to decline until the early 2000s (Magee et al. 2005; Kovach et al. 2019). Following these declines, the USFWS was petitioned to list grayling as threatened in 1991 and FWP hired dedicated staff to research grayling biology and develop management actions to address threats to the species. The Big Hole Watershed Committee was created in 1995 to address landowner concerns about grayling declines and develop strategies to prevent ESA listing, and the Big Hole CCAA program was created in 2006 to focus FWP, NRCS, DNRC, USFWS, and private landowner resources towards developing grayling conservation strategies on private lands and providing assurances against additional regulations if grayling become listed (USFWS 2006). Following drainage-wide conservation measures to improve instream flows, riparian health, fish passage, and entrainment in irrigation diversions, mean N_b increased by over 160% (2012-2021) compared with past sampling (2006-2011) and genetic diversity has remained high and stable (Figure 3; Kovach et al. 2019). Currently, grayling occupy at least 100 miles of the mainstem river, select side channels, and tributaries but are most concentrated between Divide and Wisdom (FWP unpublished data). Occupied tributaries include (upstream to downstream) Steel Creek, Swamp Creek, McVey Creek, North Fork Big Hole River, Plimpton Creek, Pintler Creek, York Gulch, Fish Trap Creek, Seymour Creek, LaMarche Creek, and Deep Creek.

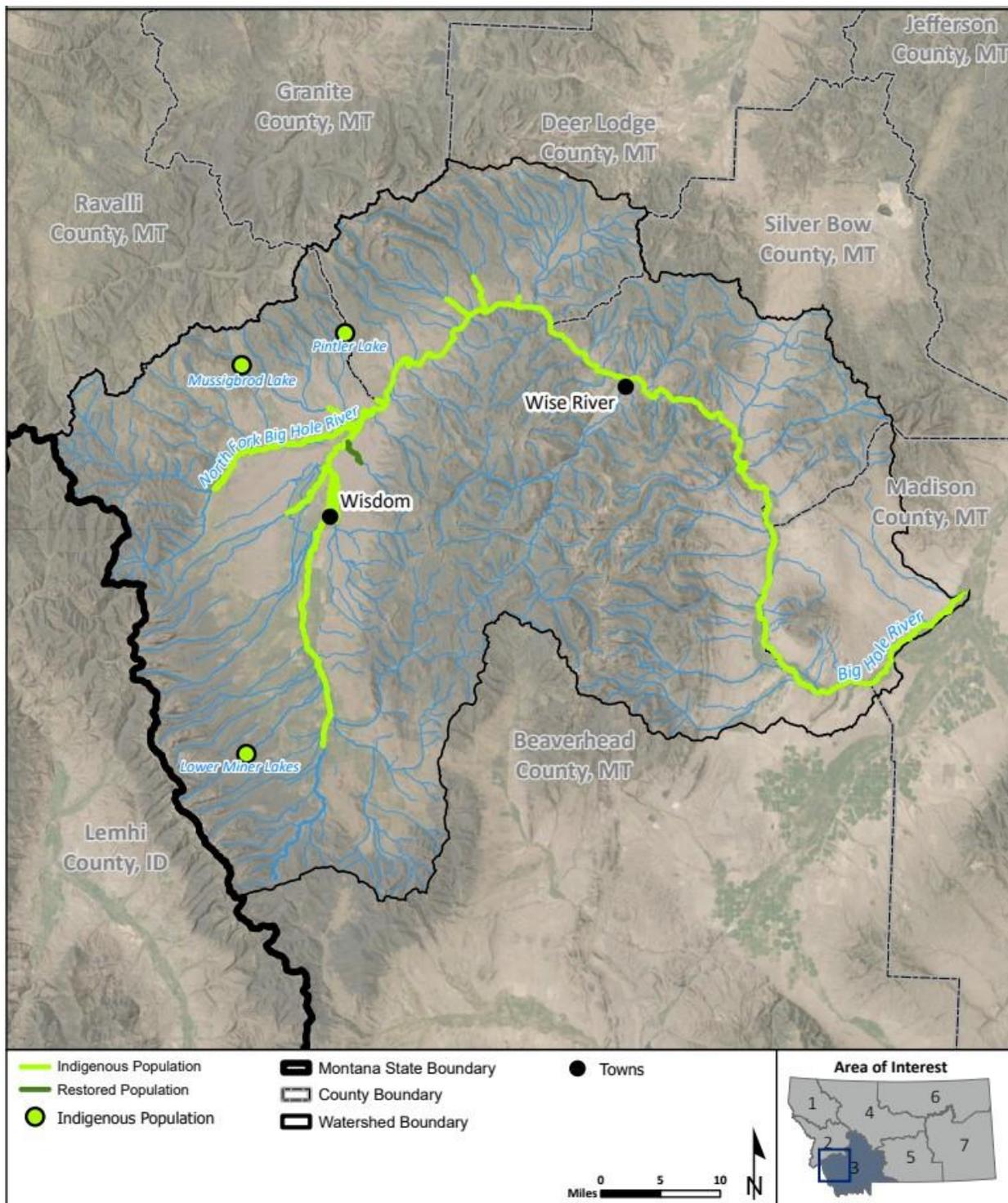


Figure 2. Distribution of Arctic grayling in the Big Hole River including three lakes with indigenous populations of grayling.

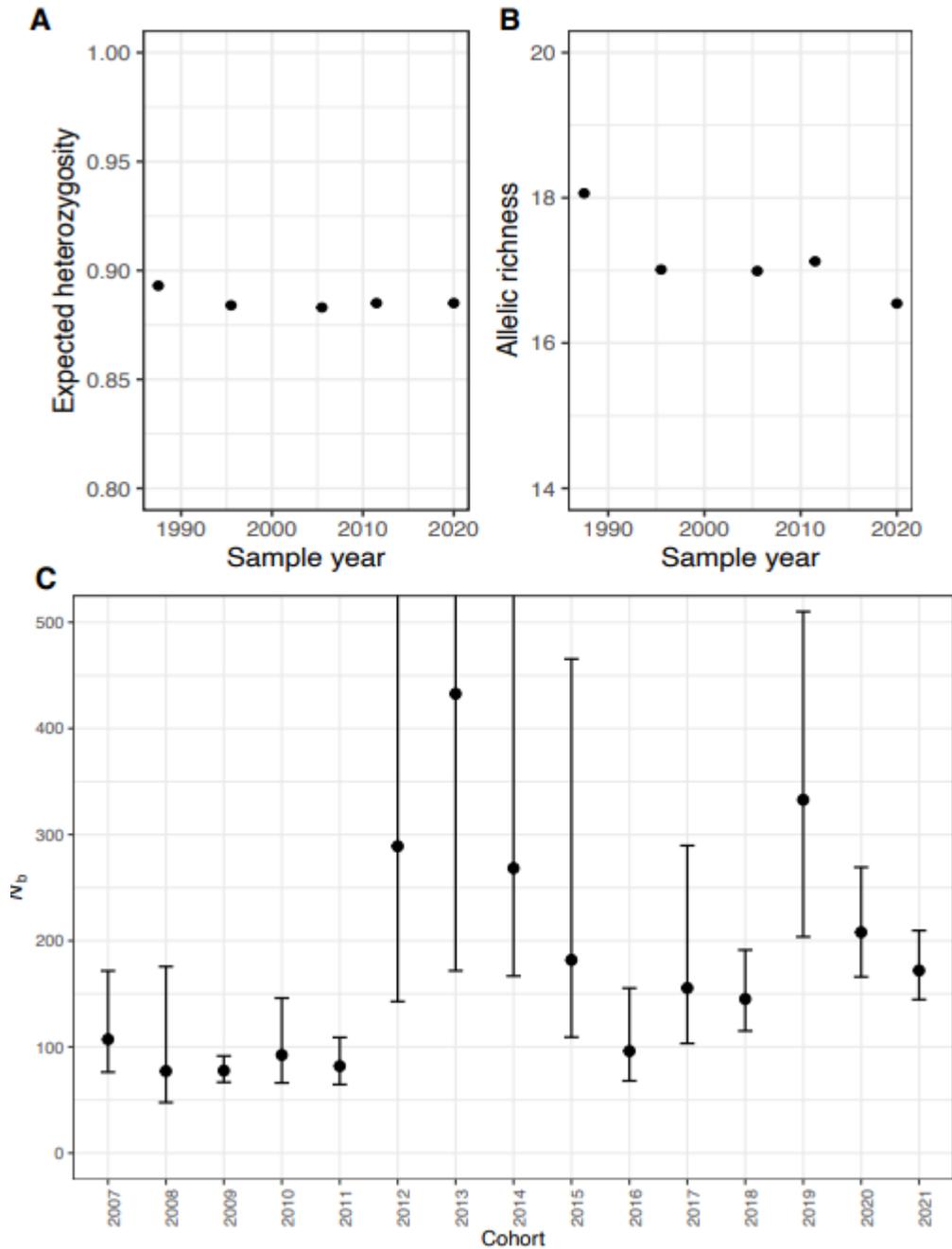


Figure 3. Genetic status of Big Hole River grayling A) Expected heterozygosity from 1990-2020, B) Allelic richness from 1990-2020, C) Number of effective breeders (N_b) of Arctic grayling from the Big Hole River from 2007-2021. Error bars are 95% confidence intervals.

The conservation strategy for Big Hole grayling is to 1) implement all conservation measures prescribed by the Big Hole CCAA program, 2) manage nonnative trout to benefit grayling, and 3) expand the distribution of grayling in conjunction with westslope cutthroat trout restoration projects within the Big Hole drainage.

Implementation of the Big Hole CCAA is the primary conservation strategy for Big Hole River grayling populations. The Big Hole CCAA explicitly addresses four threats to grayling persistence: low instream flows, degraded riparian habitat, barriers to fish passage, and entrainment in irrigation ditches (Table 3; USFWS 2006). The Big Hole CCAA has 32 landowners, over 160,000 acres of private lands, and 5,000 acres of State of Montana DNRC trust lands enrolled as of January 1st, 2022 (FWP 2021). Enrolled lands contain 245 miles of river and streams that are either occupied by grayling or contribute to grayling habitat. Enrolled landowners work with agency personnel to develop site-specific plans (SSP) for their lands (USFWS 2006). Each SSP includes conservation measures to provide adequate instream flow (i.e., irrigation management), improve riparian habitat, remove fish passage barriers, and reduce grayling entrainment in irrigation ditches. The Big Hole CCAA established instream flow targets that rely on voluntary reductions in water use by private water rights holders. Coordinated implementation efforts have helped to achieve instream flow targets 77% of the time from May 1st – October 1st since 2006 and prevented the Big Hole River from going dry during the severe drought of 2021 as it had in previous similar years (DNRC unpublished data). Additionally, drainage-wide riparian conditions, which were characterized as “at risk” when the Big Hole CCAA was created, have improved by 14% since 2006 and are now characterized as “sustainable” (USDA 2012; FWP 2021). To date, additional CCAA conservation measures include 153 instream flow improvement projects (e.g., diversions, water control or measurement structures), 84 stock water projects to conserve instream flows and improve riparian health, 71 projects involving about 100 miles of riparian fence, 17 livestock crossings, 80 fish ladders and 15 grade-control structures to improve passage, and 43 stream restoration projects to improve grayling habitat across 40 miles of stream channel (FWP 2021). In 2020, the USFWS found that grayling did not warrant listing under the ESA specifically because of increasing abundances and habitat improvements resulting from the Big Hole CCAA (USFWS 2020). Implementation of all SSP’s and the conservation actions contained therein is expected to result in stable or increasing N_b , N_e , A_r , and H_e and to maintain or increase grayling distribution in all reaches of the Big Hole CCAA (Table 3). Implementation of this strategy will satisfy Goal 1 by maintaining or increasing N_e and Goal 2 by maintaining or increasing the geographic distribution of the population.

Managing nonnative fish is a secondary strategy for Big Hole grayling conservation. Nonnative brook, rainbow, and brown trout are well-established with locally abundant grayling populations throughout the Big Hole River and its tributaries. Although nonnative brook trout do not appear to affect habitat use or growth of juvenile Arctic grayling (Byorth and Magee 1998), a negative relationship exists between brown trout and grayling abundances (Kaya 1992; McCullough 2017). Long-term sampling near Jackson indicated a shift in the fishery from predominantly brook trout to brown trout over the past 15 years (McCullough 2017), which could lead to changes in the grayling population. Therefore, fishing regulations to encourage the harvest of nonnative trout were established for the Big Hole River as a conservation measure to protect grayling. The entire Big Hole River is catch-and-release for grayling and the brook and brown trout creel limits are 20 and 5, respectively, with no size restrictions upstream of Dickie Bridge (FWP 2022). A potential study design to evaluate the effects of nonnative trout interactions with grayling could include the suppression of nonnative trout in select tributaries that are known to be used by grayling while maintaining nonnative populations in other streams. If nonnative trout are a significant

limiting factor of grayling distributions and abundances, management options for reducing or eliminating nonnative trout may be limited given their wide distribution and the social tolerance of management actions that might reduce popular sport fisheries. Implementation of this strategy will facilitate achievement of Goals 1 and 2.

The distribution of Big Hole River grayling population will be expanded by experimental introductions to tributaries in conjunction with westslope cutthroat trout (WCT) restoration projects. The WCT Conservation Strategy for the Missouri River Headwaters of Southwest Montana (FWP 2022b) outlines potential projects to restore WCT over large reaches of Big Hole River tributaries not presently occupied by grayling. These projects involve isolating WCT from nonnative species by constructing fish barriers and removing nonnative fish. Efforts to introduce grayling to tributaries of the Big Hole River where nonnative fish populations are abundant have largely been unsuccessful (Olsen 2020); however, when grayling were experimentally introduced in McVey Creek after nonnative fish removal, grayling became abundant and widespread. Therefore, streams, where nonnative species have been removed, may provide better opportunities for successful grayling reintroduction. Grayling will next be experimentally introduced in French Creek, where nonnative trout were removed from 40 miles of stream. The potential projects outlined in the WCT Conservation Strategy for the Missouri River Headwaters of Southwest Montana (FWP 2022b) will provide additional opportunities for UMR grayling. Implementation of this strategy will facilitate achievement of management Goals 2 and 3.

Supplementation of the Big Hole River grayling population will only occur if N_e declines below 50 for one or more generations. The grayling brood in Axolotl Lake would act as the source populations for supplementation. The Axolotl brood population is managed as a genetic reserve, but its genetic variation is lower than the current the Big Hole River population; therefore, supplementation should only occur as a last resort (Leary 1991). If necessary, implementation of this strategy would contribute to the achievement of Goal 1.

Table 3. Actions to achieve the conservation strategies for Big Hole River Arctic grayling. Participating agencies or organizations are Montana Fish, Wildlife & Parks (FWP), U. S. Fish and Wildlife Service (USFWS), Montana Department of Natural Resources (DNRC), USFWS Partners for Fish and Wildlife Program (PFWLP), and USFWS Ecological Services.

Waterbody	Conservation Measure	Lead Agencies	Cost
Big Hole and Tributaries	200 days/year flow compliance & monitoring	FWP, DNRC	\$25,000 operations & 2 existing staff
Big Hole Ditches	Improve functionality of headgates (~4 per year)	FWP, USFWS	\$40,000 operations & 2 existing staff
Big Hole Ditches	Modify existing irrigation infrastructure (~ 2/year)	FWP, USFWS	\$10,000
Big Hole Ditches	7-10 miles entrainment surveys annually	FWP	\$2,500 operations & 2 existing staff
Big Hole Ditches	Install one fish screen every five years as needed	FWP	\$150,000
Spawning Tributaries	Collect at least 120 YOY grayling genetic samples annually	FWP	\$1,000 operations & 3 existing staff
Big Hole and Tributaries	Install fish ladders or step pools (~ 3/year)	FWP, USFWS	\$5,000
Big Hole and Tributaries	Remove perched/nonfunctioning culverts (~ 1/year)	FWP, USFWS	\$5,000
Big Hole and Tributaries	Install bridges (~ 1/3 years)	FWP, USFWS	\$25,000
Big Hole and Tributaries	Install rock grade control diversions (~ 1/year)	FWP, USFWS	\$10,000
Big Hole and Tributaries	Complete 70 miles of riparian assessments on enrolled property	FWP	\$5,000 operations & 2 existing staff
Big Hole and Tributaries	Update and modify grazing plans (~ 6/year)	FWP	2 existing staff
Big Hole and Tributaries	Livestock stock water projects (~ 2/year)	FWP, PFWLP	\$30,000
Big Hole and Tributaries	Fencing for riparian pasture management (~ 3 miles/year)	FWP, CVA	\$10,000 operations & 3 existing staff
Big Hole and Tributaries	Streambank and channel restoration (~ 0.75 miles/year)	FWP, PFWLP	\$20,000

Centennial Valley: An indigenous grayling population resides in the Centennial Valley, which includes all tributaries of the Red Rock River and associated drainages upstream of Lima Dam (Figure 4). Landownership is divided among USFWS Red Rock Lakes National Wildlife Refuge (12%; Refuge), Beaverhead-Deerlodge National Forest (17%; USFS), DNRC (18%), BLM (26%), and private (27%). Centennial Valley geology (Sonderegger 1981), hydrology (Deeds and White 1926; FWP 1989; Montana Code Annotated 2000), fisheries (Nelson 1954; Randall 1978; Gillin 2001; Oswald et al. 2008; Gander et al. 2019a), and contemporary administrative status (USFWS 2009) are well-described elsewhere. Several historical summaries of Centennial Valley aquatic resources (Randall 1978; Gillin 2001), and specifically grayling (Vincent 1962; Unthank 1989), have been assembled. Centennial Valley grayling have been the focus of propagation (Henshall 1906; Kaeding and Boltz 1999; Gander et al. 2019a), management and conservation (Nelson 1954; Boltz 2000; Warren and Jaeger 2017), and research (Lund 1974; Gangloff 1996; Mogen 1996; Katzman 1998; Levine 2007; Paterson 2013; Davis 2016; Gander et al. 2019a) efforts in the Centennial Valley for over 100 years.

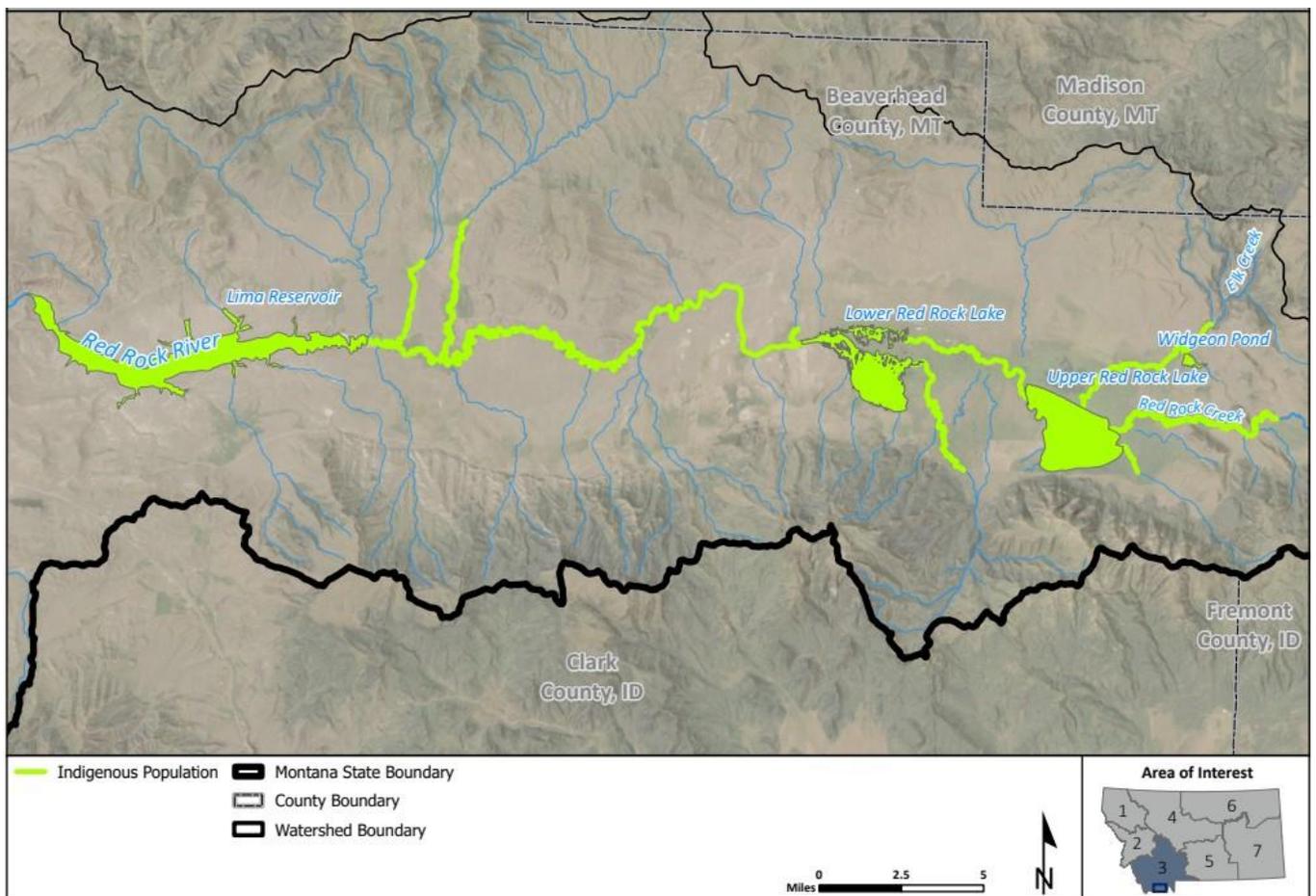


Figure 4. Arctic grayling in the Centennial Valley including the extirpated population in Elk Lake.

The distribution of Centennial Valley grayling has contracted and expanded over the past 100 years. The exact historical distribution is unknown, but most of the suitable Centennial Valley tributaries and downstream portions of the Red Rock and Beaverhead watersheds were occupied by grayling (Evermann 1893; Nelson 1954). Elk Springs (Henshall 1906) and Red Rock (Nelson 1954) creeks likely historically supported the highest abundances and most frequent use by

grayling among Centennial Valley streams. Grayling remained relatively abundant and presumably widely distributed throughout the Centennial Valley until 1930 when a major decline in distribution occurred coincident with drought and chronic valley-wide dewatering; grayling that had previously occurred in streams year-round now only occupied Upper Red Rock Lake (Upper Lake) outside of spawning periods (Harding 1915; Deeds and White 1926; Vincent 1962). Failure of grayling to return to previous distributions and abundances during improved hydrologic conditions prompted the first extensive and focused fisheries survey of Centennial Valley waters in 1951, which documented grayling in 11 streams and 2 lakes (Nelson 1954). From the 1950s to 1990s, disparate sampling assessed and described the distribution of Centennial Valley grayling distribution. By 1999, the distribution declined to only two tributaries (Red Rock and O'dell creeks) and two lake populations (Upper and Lower Red Rock lakes; (Lund 1974; Myers 1977; Randall 1978; USFWS 1985; Unthank 1989; Mogen 1996; Boltz 2000). Electrofishing and gill netting surveys of historically occupied waters between 2010 and 2013 showed grayling were present in seven streams (Red Rock, Elk Springs, Odell, Corral, Picnic, Tom, and Long creeks) and three lakes (Upper Red Rock and Elk lakes and Lima Reservoir; Gander et al. 2019a).

Centennial Valley grayling abundance and genetic variation have significantly declined in the past six years. Most Centennial Valley grayling spawn in Red Rock Creek and occupy Upper Lake the rest of the year, thus population status is assessed in Red Rock Creek during spawning periods using demographic and genetic population surveys (Paterson 2013; Gander et al. 2019). The grayling population significantly declined following near anoxic conditions in Upper Lake during the winter of 2016 and is presently at historically low abundances (Figure 5; Warren et al. 2022). Metrics of genetic diversity have similarly declined to historic lows, demonstrating the population is experiencing an increasingly severe genetic bottleneck (Figure 6; Kovach et al. 2019; Kovach et al. 2022). Immediate conservation intervention is necessary because the persistence of a small population size will erode the evolutionary legacy and resilience of Centennial Valley grayling and increase the likelihood of stochastically driven extirpation.

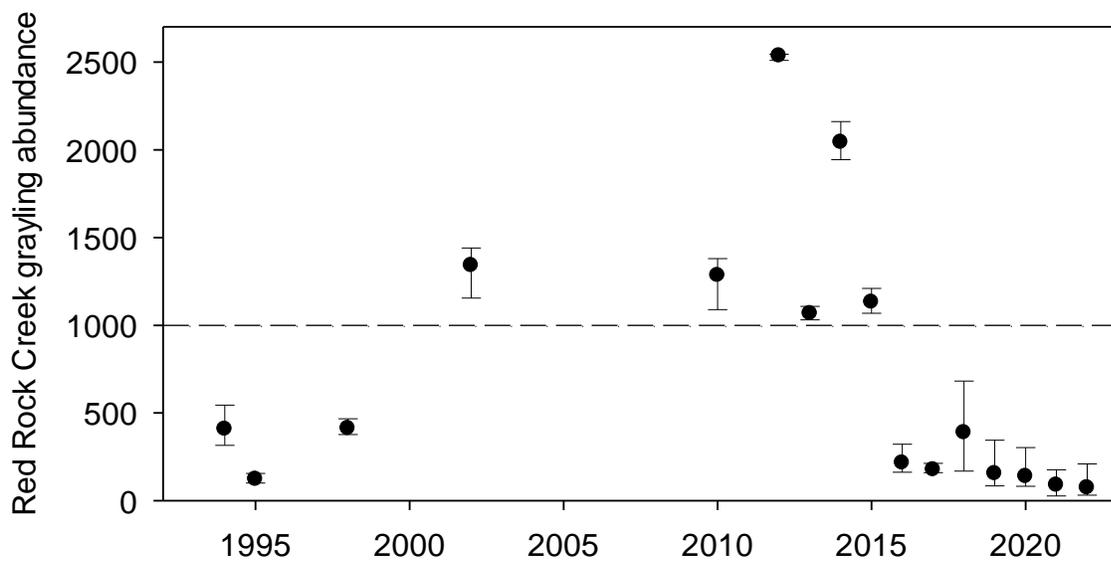


Figure 5. Abundances of spawning grayling in Red Rock Creek, 1994 to 2022. Error bars are the 95% confidence intervals. The dashed line represents the conservation goal of a population that supports at least 1000 spawning grayling.

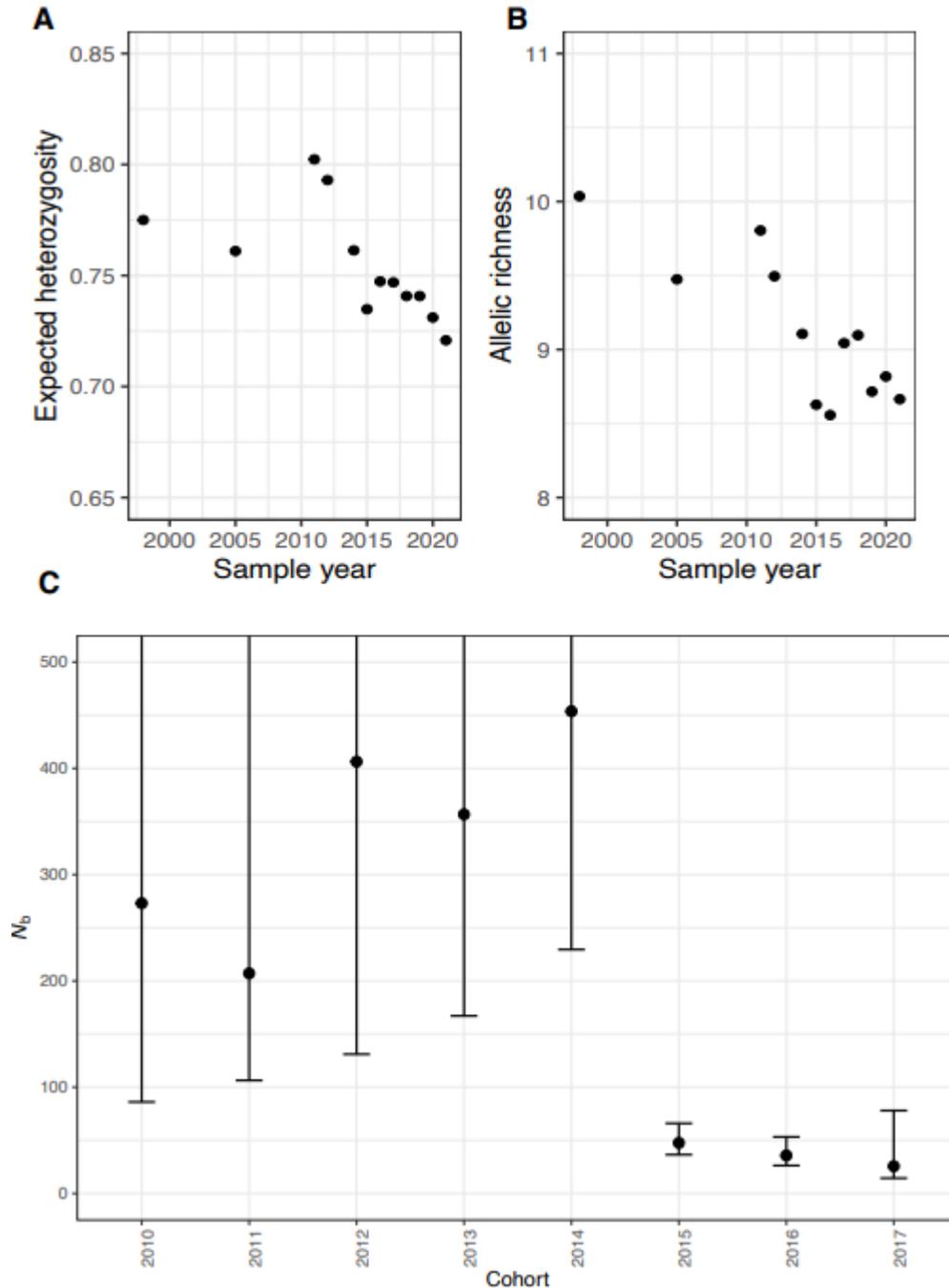


Figure 6. Genetic status of Centennial Valley grayling; A) Expected heterozygosity from 2000-2020, B) Allelic richness from 2000-2020, C) Number of effective breeders (N_b) in Arctic grayling from Upper Red Rock Lake from 2010-2017. Error bars are 95% confidence intervals.

The Centennial Valley grayling conservation strategy was developed by a stakeholder workgroup comprised of agencies and NGOs that have direct grayling or land management authority in the Centennial Valley. The workgroup met annually between 2011 and 2021 to develop and reaffirm grayling conservation measures with supporting monitoring and science. The workgroup developed three conservation strategies that will collectively satisfy the overall UMR grayling

goals and objective: 1) conserve existing Centennial Valley grayling genetic diversity, 2) establish or maintain grayling spawning and/or refugia in at least two tributaries up and downstream of Upper Lake and connectivity among tributaries, and 3) manage for at least 1000 spawning fish in the Upper Lake grayling population.

Genetic diversity of Centennial Valley grayling will be conserved by maintaining at least 1000 fish in the Upper Lake spawning population and creating a genetic reserve in Handkerchief Lake. Centennial Valley grayling genetic variation was high and stable when over 1000 fish were present in Upper Lake spawning population. However, grayling abundances have declined to historic lows following near anoxic conditions during the winter of 2016, which also led to decreases in N_b , H_e , and A_r (Figure 6; Leary et al. 2015; Kovach et al 2022). Accordingly, highest emphasis will be placed on maintaining a robust, indigenous grayling population in the Centennial Valley to achieve this strategy, which will allow it to respond to native selective forces and most effectively build inherent, long-term resiliency against extirpation. Because this conservation strategy has not been achieved recently, a genetic reserve (brood) of Centennial Valley grayling is being created in Handkerchief Lake by amalgamating stocked lake populations that most closely represent the ancestral Centennial Valley genetic legacy (see *Handkerchief Lake* in *Genetic Reserve* section below; Kovach 2021). The brood may be used to infuse genetic variation or restore Centennial Valley grayling to streams where they have been extirpated. Because founding populations were isolated and genetically drifted from the Centennial Valley population, use of this brood would only occur under extreme circumstances (e.g., genetic variability of extant population was lower than the brood) and as a last resort. Establishing a second CV brood in Elk Lake will also be pursued. Elk Lake historically contained an indigenous population of grayling which was extirpated in the 1990s when connectivity to its two spawning tributaries was eliminated. In 2011, a project was initiated on Narrows Creek (tributary to Elk Lake) to restore surface flow sufficient to support spawning by and re-establishment of self-sustaining Arctic grayling and WCT. This included installation of an underground pipeline with flow control valves from Narrows Creek Reservoir to the lower portion of an adjacent perennial spring creek, and habitat restoration work in the spring creek to improve quality of spawning habitat. The total cost for this project was \$71,000. Although the project was successful in providing increased spawning by WCT, the current storage capacity of the reservoir was not adequate to provide a benefit to grayling which generally spawn 2-3 weeks later than WCT. FWP and the USFS will continue to pursue opportunities to restore grayling to Elk Lake including increasing the capacity of Narrows Creek Reservoir and horizontal drilling to increase flow in the spring creek from 0.5 CFS to 1.2 CFS.

Maintenance and restoration of spawning and refugia habitats will be prioritized upstream of Upper Lake in Red Rock and Elk Springs creeks. Red Rock and Elk Springs creeks occur primarily on Refuge lands and have received considerable conservation focus. The Red Rock Compact and Jefferson River Basin closure ensures instream flows on Red Rock Lakes tributaries are maintained (Montana Code Annotated 2000). A Comprehensive Conservation Plan stipulates numerous actions to benefit Arctic grayling and their habitats on the Refuge, including restoring formerly occupied Arctic grayling habitat and minimizing deleterious effects of grazing, dewatering, and habitat degradation (USFWS 2009). A CCAA was developed for Centennial Valley grayling that addresses riparian habitat, entrainment, fish passage, and instream flows on private lands (USFWS 2018). Conservative angling regulations (e.g., catch-and-release, spawning closure

from May 1-June 15) are in place on both streams (FWP 2022). Elk Springs Creek restoration included MacDonald Pond removal in 2009, direct reconnection to Upper Lake in 2016, and headwaters and former lakebed spawning habitat restoration in 2016 and 2021, respectively. The restoration of the headwaters of Red Rock Creek and tributary habitat occurred in 2010 and 2020, respectively. As part of CCAA site-specific plans, irrigation diversions were replaced and measuring devices were installed to improve water control and fish passage. Additional habitat restoration and CCAA site-specific and grazing plan development are needed on Red Rock Creek, but this conservation strategy will otherwise be satisfied through annual monitoring of the grayling population, fish barriers, and flow compliance in addition to periodic entrainment monitoring (Table 4; Warren and Jaeger 2017; USFWS 2018; Warren et al. 2019).

Arctic grayling spawning and refugia habitat downstream of Upper Lake will be prioritized in Long, Middle, and West creeks. Long, Middle, and West creeks occur almost entirely on private lands. The Centennial Valley CCAA directly addresses the primary threats to grayling spawning and refugia habitat on those streams (degradation of riparian habitats, entrainment, fish passage, and instream flows; USFWS 2018). Restoration projects to improve riparian habitats, floodplain connectivity, and spawning and rearing habitats occurred between 2010 and 2021 on Long Creek. Reaches of Middle Creek captured by irrigation ditches were restored to their channels in 2019. Four irrigation diversions and five measuring devices were replaced to improve water control and fish passage among the streams and a hardened livestock crossing was installed on West Creek to improve riparian habitats between 2019 and 2021. Three headgates, measuring devices, and irrigation diversion modifications to improve fish passage and water control, one stock tank, about 0.5 miles/year of stream restoration, one grazing plan, two CCAA site-specific plans, and 40 days/year flow and compliance monitoring are needed to fulfill this conservation strategy (Table 4). Periodic population and entrainment monitoring is also required (Table 4; USFWS 2018).

The Centennial Valley Arctic Grayling Adaptive Management Plan (AMP) will be implemented to manage for at least 1,000 spawning fish in the Upper Lake grayling population. The AMP is a tool intended to facilitate achievement of Centennial Valley grayling conservation strategies in perpetuity (Warren and Jaeger 2017). The AMP was collaboratively developed by the Centennial Valley workgroup between 2012 and 2017 to resolve structural uncertainty about population drivers and long-standing disagreement among resource managers about what actions would most effectively conserve grayling (Warren and Jaeger 2017). The workgroup set a threshold for management intervention at 1,000 spawning fish using an expert elicitation process that defined the population size where long-term self-sustaining persistence of grayling was expected (Boyd 2014). If the spawning population is less than 1,000 fish, management actions predicted to restore the population to the target most quickly will be implemented (Warren and Jaeger 2017). The AMP investigated 1) reduction and alteration of spawning habitat, 2) predation by and competition with nonnative fishes, and 3) limited winter habitat in Upper Lake as potential population drivers by implementing sequential, large-scale management experiments. The amount of suitable overwinter habitat in Upper Lake was identified as, and continues to be, the primary population driver; a threshold level of 10–25 ha of habitat > 1 m deep and > 4mg/L O₂ appears necessary to overwinter 1,000 grayling (Warren et al. 2022). An alternatives analysis was completed in 2019 to assess costs, logistical and legal feasibilities, and likely effects on grayling of winter habitat enhancement approaches (Flynn et al. 2019). Following piloting of several

alternatives, the potential options to create oxygenated deep-water habitat throughout the winter are tributary outlet modification, electric aeration, and building a berm to route Elk Springs Creek effluent to deep parts of the lake (Flynn 2022). Selection and implementation of management alternatives to improve overwinter habitat in Upper Lake is necessary to fulfill this strategy and the overall conservation objective for Centennial Valley grayling; the other two conservation strategies are contingent on this strategy being successful to collectively preserve long-term self-sustaining persistence of grayling.

Table 3. Measures to achieve Centennial Valley Arctic grayling conservation strategies. Participating agencies or organizations are Montana Fish, Wildlife & Parks (FWP), U. S. Fish and Wildlife Service (USFWS), Red Rock Lakes National Wildlife Refuge (Refuge), USFWS Partners for Fish and Wildlife Program (PFWLP), USFWS Ecological Services (ES), Centennial Valley Association (CVA), The Nature Conservancy (TNC), and Montana Trout Unlimited (TU).

Waterbody	Conservation Measure	Lead Agencies	Cost
Red Rock Creek	At least 1.5 miles/year population surveys	FWP	\$4,000 operations & 3 existing staff
Red Rock Creek	11 miles/year provide fish passage at beaver dams	FWP, Refuge	\$1,000 operations & 3 existing staff
Red Rock Creek	1 mile stream restoration	FWP, PFWLP	\$500,000
Red Rock Creek	1 CCAA site-specific and grazing plans	FWP, ES	Existing staff (5 FTE)
Red Rock Creek	15 days/year flow compliance & monitoring	FWP, CVA	\$5,000 operations & 3 existing staff
Red Rock Creek	5 miles entrainment surveys every 10 years	FWP	\$1,000 operations & 2 existing staff
Elk Springs Creek	1 mile/year population surveys	FWP	\$2,000 operations & 3 existing staff
Long, West, Middle Cr	2 miles population surveys every 5 years	FWP	\$2,000 operations & 3 existing staff
Long, West, Middle Cr	0.5 miles stream restoration/year	FWP, PFWLP, TNC	\$40,000
Long, West, Middle Cr	2 CCAA site-specific plans	FWP, ES	5 existing staff
Long, West, Middle Cr	10 miles/year riparian assessments	FWP	\$1,000 operations & 2 existing staff
Long, West, Middle Cr	1 grazing plans/year	FWP	2 existing staff
Long, West, Middle Cr	1 stock tank	FWP, PFWLP	\$30,000
Long, West, Middle Cr	40 days/year flow compliance & monitoring	FWP, CVA	\$10,000 operations & 3 existing staff
Long, West, Middle Cr	3 headgates	FWP, PFWLP	\$20,000
Long, West, Middle Cr	3 diversion passage modifications	FWP, PFWLP	\$20,000
Long, West, Middle Cr	5 miles entrainment surveys every 10 years	FWP	\$1,000 operations & 2 existing staff
Upper Red Rock Lake	10-25 ha habitat >1 m deep and > 4mg/L O ₂	FWP, Refuge, ES, TU	\$650,000
CV AMP	Substrate and winter habitat monitoring, data analysis, reporting	FWP, Refuge, CVA, TNC	\$10,000 operations & 8 existing staff

Miner, Mussigbrod, and Pintler Lakes: Miner, Mussigbrod, and Pintler lakes in the Big Hole River drainage support native populations of grayling (Figure 2). Although each lake was stocked from the 1930s through the early 1950s with millions of grayling that were likely of Red Rock Lakes origin, genetics analyses indicate the grayling populations in these lakes are predominantly of Big Hole River ancestry (Peterson and Arden 2009; Leary et al. 2015; Kovach et al. 2021). Nonnative brook trout are common in the lakes as well as several native species including burbot *Lota lota*, longnose sucker *Catostomus catostomus*, and white sucker *Catostomus commersonii*.

Mussigbrod Lake is the largest (116 acres) and one of the deepest (> 70 ft) lakes in the Big Hole drainage. Historically, Mussigbrod Creek was well-connected to Mussigbrod Lake; however, a dam across the historical outlet of the lake was constructed, which raised lake elevation 10-15 ft and moved the outlet several hundred feet to the west at full pool. The new outlet is a concrete spillway with a 3-ft drop to a constructed boulder channel that connects to the natural stream channel. The altered outlet serves as an upstream barrier to fish passage into Mussigbrod Lake except during exceptionally high flow events. The lake surface elevation typically decreases to its historical level as irrigation demand increases in the summer months; however, the lake often refills by the following June. Despite habitat modifications and lake elevation fluctuations, effects on grayling have been negligible and the population remains abundant (Olsen 2014). Spawning occurs in the new outlet immediately upstream of the spillway and in other areas of the lake in mid-May. Grayling occur in high densities near the inlet of Mussigbrod Creek during the same timeframe indicating the inlet stream may also serve as spawning habitat. Grayling are rarely captured in Mussigbrod Creek upstream and downstream of the lake outside of the spawning season despite abundant, high-quality spawning habitats in those reaches. Oswald et al. (2007) and Olsen (2014) provide additional information dating back to the early 1970s for the Mussigbrod Lake grayling population.

Miner Lake is 66 acres, has a maximum depth of 34 ft (Olsen 2014), and is well-connected to Miner Creek. The lake consists of large, shallow (< 3 ft) silt flats with two distinct depressions in the upper lobe of the lake. Recent data collected at Miner Lake suggests the grayling population is abundant (Olsen 2014). Grayling spawn in the narrows between the upper and lower lobes of the lake. Although grayling have been captured in high abundances near the inlet, no spawning has been observed there. Spawning has not been documented in the outlet stream either, despite it having high-quality habitat. Similar to the Mussigbrod Lake population, grayling are rare in Miner Creek upstream and downstream of the lake. Oswald et al. (2007) and Olsen (2014) provide additional information dating back to the early 1960s for the Miner Lake grayling population.

Pintler Lake is the smallest of the three lakes that support native grayling populations in the Big Hole River drainage. The lake is about 39 acres with a maximum depth of 21 ft. Although depths exceed 10 ft near the inlet, about 75% of Pintler Lake is < 4 ft deep with extensive silt flats and aquatic macrophytes. Above the lake, Pintler Creek flows through a large wetland with extensive beaver activity that may restrict fish passage. Pintler Falls, located approximately 1.3 miles upstream of Pintler Lake, is a complete upstream barrier to fish passage. The stream below the lake is low gradient and highly sinuous with abundant high-quality spawning habitat. Sampling in 1964, 2006, and 2009 indicated that suckers are the most common fish in Pintler Lake followed by grayling and brook trout (Olsen 2014). The average size of grayling in Pintler Lake is greater

than that observed in either Miner or Mussigbrod lakes with some fish approaching 15 inches. Attempts to collect adequate grayling samples (30 fish) from Pintler Lake have been less successful, and samples submitted in 2022 were the result of multiple capture events over three years.

The conservation strategy for indigenous grayling populations in the three Big Hole lakes is to periodically monitor genetic diversity of the populations and supplement (i.e., genetic rescue) as necessary. The genetic variation of the grayling populations in Mussigbrod, Miner, and Pintler lakes will be monitored to ensure that N_e is remaining stable or increasing. Genetic variation of the Miner and Mussigbrod grayling populations is stable (Table 5), a result of high grayling abundances and suitable habitats in both lakes. An updated genetic assessment of Pintler Lake grayling is needed to determine the status of that population (samples submitted in 2022) (Table 5). Effective implementation of this strategy will ensure that the three overall management goals for UMR grayling are achieved.

If future declines in genetic variation are observed, lake-specific conservation strategies will be developed based on factors limiting the population. Conservation strategies would attempt to improve genetic variation by implementing actions likely to increase grayling abundances and/or simulate geneflow by translocating grayling from one of the other two native Big Hole lake populations.

Replicating the Miner, Mussigbrod and potentially Pintler lakes grayling populations in Twin Lakes is a secondary conservation strategy. Twin Lakes is a low-elevation lake in the Big Hole River drainage that was recently occupied by grayling prior to 1900 (Shields 1897) and is also home to a native population of lake trout *Salvelinus namaycush*. About 150,000 eggs from Mussigbrod Lake and 50,000 eggs from Miner Lake were incubated in the narrows between Twin Lakes in 2017 and 2019. No subsequent monitoring of the lake has been completed, so the success of the introduction is presently uncertain. If monitoring indicates the previous introduction did not produce a self-sustaining population, native Big Hole lakes grayling will be stocked at a density of 1,500-2,000 fish per acre for at least a three-year period. If the Twin Lakes introduction is unsuccessful, 1-2 populations will be established in lakes elsewhere in the Big Hole River drainage. This conservation measure will increase the geographic distribution of native Big Hole lakes grayling and replicating these populations will create a genetic reserve that could be used for subsequent grayling introductions or to improve genetic variation of the native Big Hole lake populations as necessary. Big Hole lakes conservation measures are expected to cost \$1,000 to \$10,000 each and will be completed by existing FWP staff (Table 6). Implementation of this strategy will facilitate achievement of all three overall management goals for UMR grayling.

Table 4. Genetic metrics of Arctic grayling from native Big Hole River lakes (H_e = average heterozygosity; A_r = allelic richness; N = sample size).

Population	Year	H_e	A_r	N
Miner Lake	2020	0.81	9.26	41
Miner Lake	2016	0.80	9.36	58
Miner Lake	2006	0.80	9.24	37
Mussigbrod Lake	2020	0.75	7.50	32
Mussigbrod Lake	2012	0.75	7.69	50
Mussigbrod Lake	2006	0.75	7.88	48
Pintler Lake	2009	0.67	4.79	32

Table 6. Measures to achieve Mussigbrod, Miner and Pintler lakes grayling conservation strategies. Participating agency is Montana Fish, Wildlife & Parks (FWP).

Waterbody	Conservation Measure	Lead Agency	Cost
Mussigbrod Lake	Monitoring every 6-8 yrs	FWP	\$1,000 operations & 3 existing staff
Miner Lake	Monitoring every 6-8 yrs	FWP	\$1,000 operations & 3 existing staff
Pintler Lake	Monitoring every 6-8 yrs	FWP	\$1,000 operations & 3 existing staff
Twin Lakes	Monitoring, additional introductions	FWP	\$10,000 operations & 5 existing staff

Ruby River: Grayling were reintroduced to the Ruby River to establish a stable, naturally reproducing population (Byorth 1996). The upper Ruby River above Ruby Reservoir (Ruby River) was selected for grayling reintroduction because of the size (\approx 41 unfragmented miles of habitat), low gradient (mean = 0.7%), abundant pool habitat, and low density of nonnatives (Kaya 1992; Liermann 2001). Grayling were initially reintroduced by stocking fry and fingerlings from 1997 to 2005; however, the subsequent use of remote site incubators from 2003 to 2008 had better results (Gander et al. 2019b). The population was deemed a self-sustaining UMR conservation population when natural reproduction, without supplementation, was documented for 10 consecutive years and eDNA results indicated a wide distribution (Gander et al. 2019b). Arctic grayling currently inhabit the majority of the mainstem Ruby River above Ruby Reservoir (Figure 7). Post-stocking measures of genetic diversity (H_e and A_r) were relatively high and stable from 2010 to 2018; however, declines in N_b suggest that periodic monitoring is warranted to avoid loss of genetic diversity in future generations. Future planned actions to benefit grayling in the Ruby River include active stream restoration to reduce sediment inputs, increase floodplain connectivity, improve instream habitat and riparian health on public and private land in the Ruby Valley.

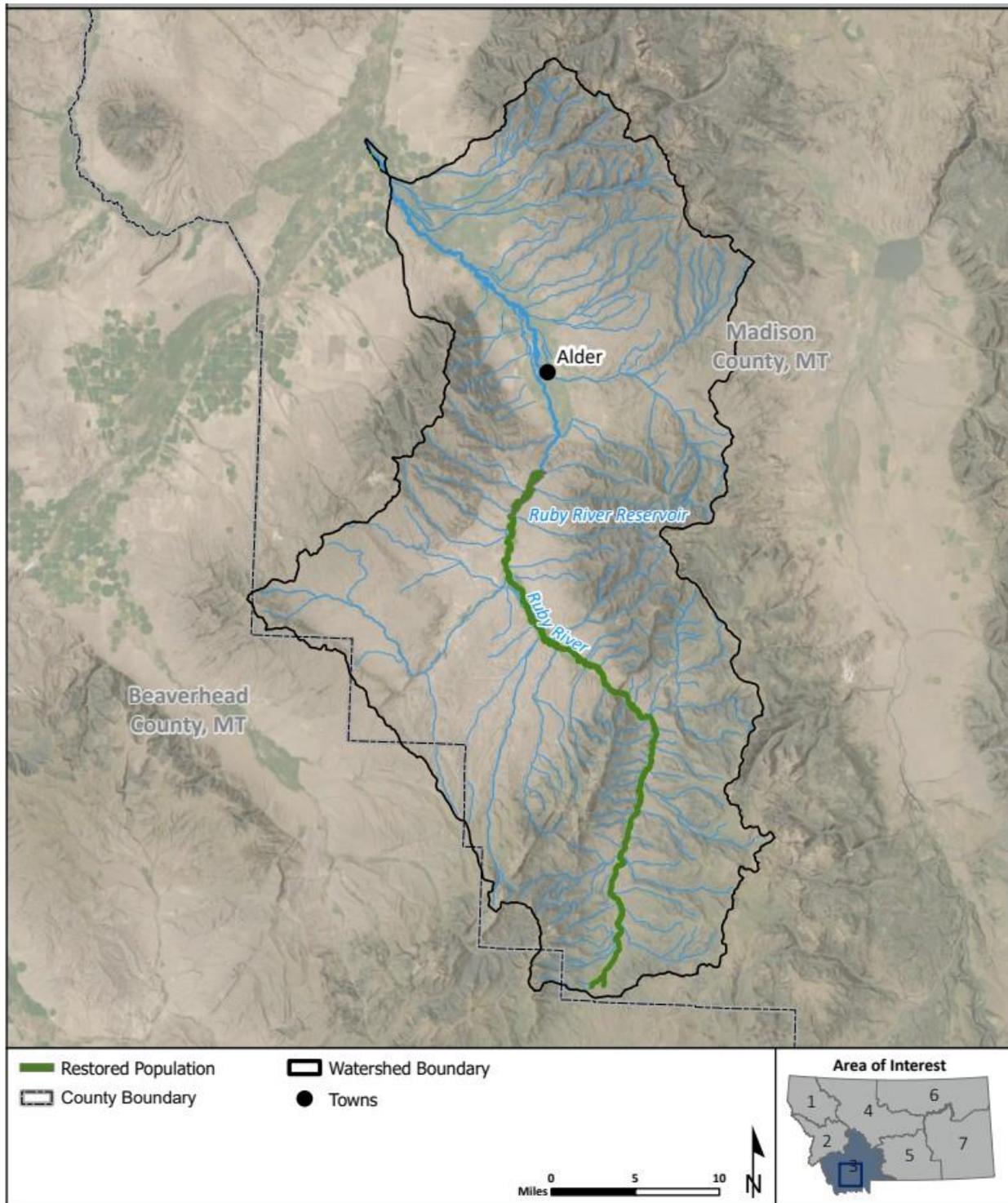


Figure 7. Distribution of the reintroduced Arctic grayling population in the upper Ruby River.

Table 7. Genetic monitoring results for grayling in the upper Ruby River, 2010 to 2018. Non-parenthesized sample sizes refer to mixed-age samples used to estimate average expected heterozygosity (H_e) and allelic richness (A_r) in a given year. Parenthesized samples refer to the number of samples from a given cohort used to estimate effective number of breeders (N_b).

Year	H_e	A_r	N_b	Sample Size
2010	0.7930844	7.804212	23.5 (15.8, 36.9)	25 (48)
2011	0.8294469	9.054225	24.8 (12.6, 86.1)	27 (19)
2012	0.8554041	9.608342	42.1 (17.2, Inf.)	27 (20)
2013	NA	NA	NA	NA
2014	NA	NA	20.2 (11.6, 38.8)	NA (38)
2015	0.8573938	9.438658	13.4 (7.0, 26.1)	19 (36)
2016	0.8588745	9.59538	NA	28
2017	0.8244626	8.000473	6.9 (3.9, 9.8)	78 (76)
2018	0.8502296	8.903817	NA	33

The conservation strategy for the Ruby River grayling population is to monitor genetic variation to ensure a stable or increasing genetic effective population size. If future declines in genetic variation are observed, conservation measures would include genetic infusion by introducing embryos via remote site incubators at similar locations and densities as during grayling introductions (Gander et al. 2019b). If necessary, additional supplementation the Ruby River grayling population will be monitored next in 2023 by FWP and USFS staff (Table 8). Implementation of this strategy will ensure that overall management goals 1-3 for UMR grayling are achieved.

Table 8. Measures to achieve the Ruby River grayling conservation strategy. Participating agencies are Montana Fish, Wildlife & Parks (FWP) and the U.S. Forest Service (USFS).

Waterbody	Conservation Measure	Lead Agency	Cost
Ruby River	Monitoring every 6-8 yrs	FWP, USFS	\$5,000 operations & 6 existing staff

Madison River: Grayling were historically widely distributed and abundant in the Madison River but are now believed to be extirpated. The decline of grayling in the Madison River was more gradual than in neighboring drainages (e.g., Gallatin, Sun, Smith rivers) because land and water use was initially less severe in the Madison River drainage (Vincent 1962). The construction of Ennis and Hebgen reservoirs in 1901 and 1914, respectively, eliminated many spring creek complexes that served as the primary spawning areas for grayling in the Madison River. Specifically, Horsethief Springs, Grayling Creek, and South Fork Madison River contained abundant grayling populations prior to the construction of Hebgen Dam (Vincent 1962). Grayling were common throughout the Madison River between Ennis Dam and Hebgen Dam until the 1950s (Kaya 1990) but declined to a small remnant population near Ennis Reservoir by 1980 (Byorth and Shepard 1990). High densities of nonnative trout in the mainstem Madison River and many tributaries are believed to limit current grayling restoration efforts and compound the effects of historic inundation of spawning habitat by Hebgen and Ennis dams. Ultimately, both factors have contributed to their extirpation (Vincent 1962). Although Arctic grayling in the Madison River are occasionally reported by anglers, the lack of detection of grayling during standardized fisheries sampling indicates indigenous grayling have likely been extirpated from the drainage and only recently reintroduced fish exist at abundances too low to support a self-

sustaining population. Grayling in the Madison River are now considered to be reintroduced populations with reintroduction efforts ongoing (Figure 8).

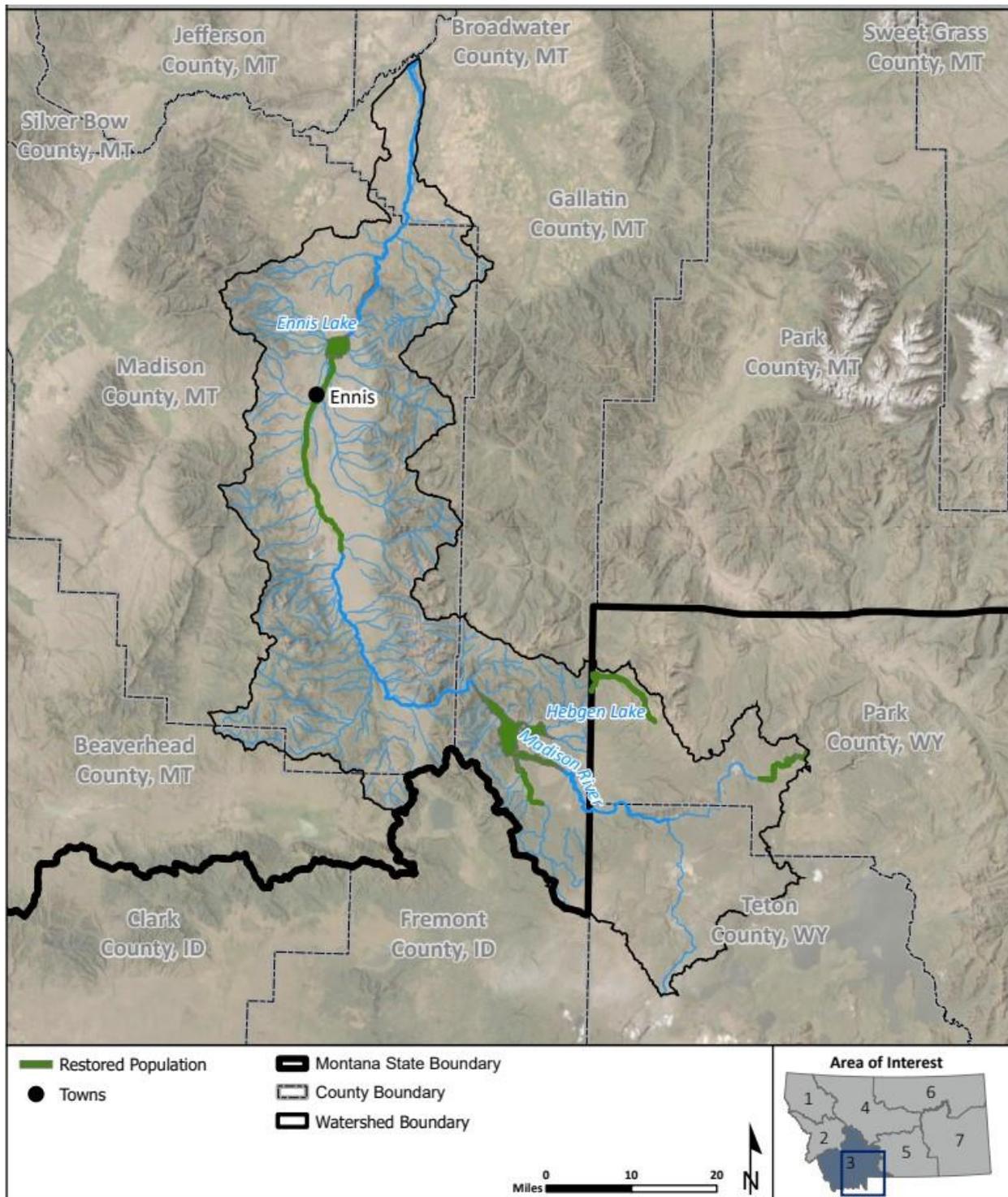


Figure 8. Current distribution of reintroduced grayling in the Madison River.

The primary conservation strategy for Madison River grayling is to establish at least two viable populations in the drainage. Previous attempts to reintroduce grayling in the Madison River drainage were unsuccessful, presumably because high densities of brown trout in the tributaries

and the mainstem Madison River precluded the establishment of viable populations. Therefore, future reintroductions in the Madison River drainage will be focused on Hebgen Reservoir and its tributaries. Reintroduction of grayling in Hebgen Reservoir (5,080 hectares; Table 9) will require using at least 500,000 eggs/year for 3-5 consecutive years. (Based on available hatchery space, 500,000 is currently considered to be the annual maximum egg number possible. If capacity can increase to produce 1,000,000 eggs, we will pursue that number as a goal). Reintroductions will also occur in the upper reaches of Grayling Creek and the Gibbon River where nonnative fish have been removed, and in the South Fork Madison River where densities of resident nonnative trout are low (Table 9). Grayling from populations with primarily Madison River genetic ancestry will be used for reintroductions when possible (Kovach et al. 2021). The Axolotl Lake population may also be used to meet demographic goals. Implementation of this strategy will enable the achievement of all three overall management goals for UMR grayling.

Table 9. Measures to achieve Madison River grayling conservation strategies. Participating agencies are Montana Fish, Wildlife & Parks (FWP), Yellowstone National Park (YNP), the U.S. Forest Service (USFS), and Northwestern Energy, who funds salary for FWP staff working on grayling conservation.

Waterbody	Conservation Measure	Lead Agency	Cost
S. Fk. Madison	Introductions, monitoring	FWP, USFS	\$5,000 operations & 6 existing staff
Gibbon River	Monitoring, additional introductions	YNP, FWP	\$5,000 operations & 10 existing staff
Grayling Creek	Monitoring, additional introductions	YNP, FWP	\$5,000 operations & 10 existing staff

Gallatin River: Indigenous grayling were extirpated from the Gallatin River over a century ago but introduced grayling presently exist within a reach of the Gallatin Canyon. Grayling were historically abundant and widespread throughout the mainstem Gallatin River, spring creeks, and lower reaches of other large tributaries (Kaya 1990); however, they were probably extirpated from the Gallatin River drainage by the early 1900s because of competition and predation from nonnative species and habitat degradation associated with water withdrawals for irrigation (Vincent 1962). Grayling exist in low abundances and are primarily limited to reaches in the Gallatin River between Big Sky and Wilson Bridge near Gallatin Gateway (Figure 1). Grayling inhabiting the Gallatin River probably dispersed from Green Hollow Pond, which supports a Big Hole genetic reserve population. Angler reports have been limited to adult fish and no grayling have been captured during long-term monitoring efforts in the Gallatin River or its tributaries; therefore, it is unlikely that a self-sustaining population exists.

The primary conservation strategy for the Gallatin River is to establish grayling populations in Chiquita Lake and one additional tributary stream to increase the distribution of grayling. Chiquita Lake is a small lake (1.4 hectares) in the headwaters of the North Fork of Spanish Creek where nonnative trout were removed as part of a WCT reintroduction. Six thousand grayling fingerlings, produced from the Rogers Lake population, were released into Chiquita Lake in 2021 and 2022. Additional stocking of grayling is planned for Chiquita Lake in 2023. If reintroduction efforts in Chiquita Lake fail, a different mountain lake within the Gallatin River drainage will be pursued for reintroduction. Reintroductions in the upper Gallatin River or its tributaries will occur once reintroductions in the Madison River drainage are complete, or hatchery capacity increases to produce enough eggs and juvenile fish for larger introductions (>500,000/year). Based on historical occupancy and quality of existing habitats, the headwaters of the Gallatin River, Snowflake Springs near the Yellowstone National Park boundary, and the Taylor Fork are

potential reintroduction locations. Reintroductions will use grayling of primarily Madison genetic ancestry (Kovach et al. 2021) when possible, but supplementation with fish from Green Hollow or Axolotl lakes may be necessary to meet demographic goals. Successful implementation of this strategy will ensure the achievement of all three overall management goals for UMR grayling.

Table 10. Measures to achieve Madison River grayling conservation strategies. Participating agencies are Montana Fish, Wildlife & Parks (FWP), Yellowstone National Park (YNP), the U.S. Forest Service (USFS), and Northwestern Energy, who funds salary for FWP staff working on grayling conservation.

Waterbody	Conservation Measure	Lead Agency	Cost
Chiquita Lake	Introductions, monitoring	FWP, USFS	\$5,000 operations & 6 existing staff
Gallatin streams	Introductions, monitoring	FWP, USFS, YNP	\$5,000 operations & 6 existing staff

Mountain Lakes: Widespread, historical stocking in mountain lakes throughout Montana established 12 UMR grayling conservation populations (Figure 1; Tables 11 & 12). Introduced grayling populations that are self-sustaining and occur in lakes or reservoirs within the UMR are considered conservation populations (USFWS 2014). Viable grayling populations outside of the UMR have value as genetic reserves but are not explicitly considered as conservation populations because they occur outside of the geographic boundary of the Distinct Population Segment (DPS; USFWS 2020). Analysis of the genetic ancestry of all introduced populations, inside and outside of the DPS, identified potential donor sources for introductions in the Centennial Valley and Madison River drainages (Kovach et al 2021). Genetic monitoring also revealed a relatively stable trend in genetic variation for most established grayling populations (Table 12).

The conservation strategy for introduced UMR mountain lakes grayling populations is to monitor genetic variation to maintain a stable or increasing genetic effective population size. If future declines in genetic variation are observed, embryos, fry, or fingerlings from source populations with appropriate genetic ancestry will be used to bolster genetic variation and population size (Table 11). Successful implementation of this strategy will ensure the achievement of all three overall management goals for UMR grayling.

Table 11. Characteristics and primary genetic ancestry of introduced grayling mountain lake populations (CV = Centennial Valley; M = Madison River; RB = rainbow trout; RBxCT = hybrid cutthroat; EB = brook trout; YCT = Yellowstone cutthroat trout; WCT = westslope cutthroat trout; WSU = white sucker).

Lake Name	Drainage	Elevation (ft)	Lake Size (ac)	Total length (mm)	Other Species Present
Agnes Lake*	Big Hole	7,537	108	260-354	None
Bobcat Lake*, ^{CV}	Big Hole	8,405	6	190-305	None
Odell Lake*, ^{CV}	Big Hole	8,390	33	200-320	RBxCT, EB
Schwinegar Lake*, ^{CV}	Big Hole	8,350	4	101-305	None
Emerald Lake*	Gallatin	8,980	14	254-301	None
Grayling Lake*, ^M	Gallatin	8,360	3	208-296	None
Hyalite Reservoir*	Gallatin	6,704	158	285-431	EB, YCT
Deer Lake*, ^M	Gallatin	9,105	12	212-368	None
Park Lake*, ^{CV}	U. Missouri	6,320	32	152-305	WCT
Lake LeVale*, ^M	Sun	7,357	12	75-125	None
Gibson Reservoir*	Sun	4,798	1288	N/A	EB, RB, WCT, WSU
Grebe Lake*	Madison	8,023	156	N/A	WCT
Cliff Lake	Clark Fork	8,593	16	183-284	None
Rogers Lake ^M	Flathead	3,998	239	<406	WCT
Red Meadow Lake ^M	Flathead	5,605	16	178-279	WCT
Meadow Lake ^M	Wyoming	7,900	50	N/A	None
Elizabeth Lake ^{CV}	Belly	4,896	195	N/A	RB
Cascade Lake	Yellowstone	7,995	30	N/A	YCT

* conservation population

^{CV} primarily of Centennial Valley ancestry

^M primarily of Madison River ancestry

Table 12. Heterozygosity (H_e) and allelic richness (A_r) of introduced grayling lake populations.

Population	Year	H_e	A_r
Agnes Lake	2020	0.83	9.46
Agnes Lake	2016	0.83	9.55
Bobcat Lake	2020	0.79	6.88
Bobcat Lake	2012	0.80	7.27
Bobcat Lake	2006	0.79	7.59
Cliff Lake	2020	0.79	7.76
Deer Lake	2020	0.71	5.98
Deer Lake	2017	0.70	6.09
Elizabeth Lake	2019	0.82	8.92
Emerald Lake	2020	0.82	8.18
Emerald Lake	2013	0.81	7.91
Grayling Lake	2020	0.74	5.56
Grayling Lake	2012	0.70	5.10
Hyalite Reservoir	2020	0.79	7.24
Hyalite Reservoir	2013	0.68	5.52
Lake LeVale	2020	0.82	7.70
Odell Lake	2020	0.80	9.18
Odell Lake	2006	0.81	9.09
Park Lake	2020	0.81	8.16
Park Lake	2016	0.83	8.19
Red Meadow Lake	2020	0.81	7.43
Rogers Lake	2020	0.78	7.63
Schwinegar Lake	2020	0.81	7.77

Table 13. Measures to achieve mountain lakes grayling conservation strategy. Participating agencies are Montana Fish, Wildlife & Parks (FWP) and the U.S. Forest Service (USFS).

Waterbody	Conservation Measure	Lead Agency	Cost
Introduced Mountain Lakes	Monitoring every 6-8 yrs	FWP, USFS	\$5,000 operations & 6 existing staff

UMR Grayling Genetic Reserve Populations

The need to create a genetic reserve for Montana grayling was first identified in the 1980s when conservation actions for the Big Hole River population were initiated (Leary et al. 1991). Subsequently, two genetic reserves were created for Big Hole River grayling in the 1990s. Management goals for the Big Hole River genetic reserves are to 1) replicate the genetic diversity of the Big Hole River Arctic grayling population and 2) minimize genetic and phenotypic differentiation between the genetic reserve and wild Big Hole River populations. Initial efforts to create a genetic reserve for the Centennial Valley population prior to its decline in 2016 were unsuccessful. Attempts to create a Centennial Valley genetic reserve are ongoing using non-indigenous populations of grayling with a primary ancestry from the Centennial Valley.

Genetic reserve populations may be self-sustaining or perpetuated through onsite spawning and replacement (stocking). Initial founding of genetic reserves should occur using spawning practices that maximize parental contribution to prevent the decline of genetic diversity to levels observed in wild populations. Best known spawning practices include spawning two females with two males using a 2x2 cross (Figure 9). The eggs of two females will be split into two equal groups with a different male fertilizing each group. Eggs should be incubated separately so the number of parents successfully contributing to each year class is precisely known and contributions can be equalized (Table 7). Year classes should be marked with a unique tag so that different year classes may be crossed, which will convert year-class differences in genetic diversity to within population genetic diversity. In both stocked and self-sustaining genetic reserves, the infusion of wild genetics will be periodically necessary to prevent genetic straying. For the Big Hole River genetic reserve, it is recommended that at least 10 wild pairs of grayling are spawned and infused every 10 years (Leary 1991). For the Centennial Valley genetic reserve, wild fish infusions are not presently possible due to low population levels but will be incorporated once abundances rebound. Infused wild genes should represent 10% or less of all genes in a given year class to prevent a genetic bottleneck (Leary 1991).

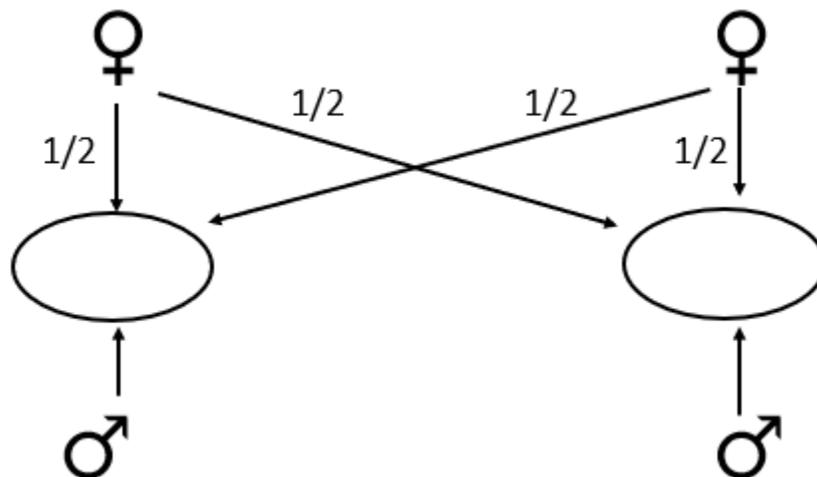


Figure 9. Schematic diagram for a typical 2x2 spawning cross.

Axolotl Lake: A Big Hole River grayling genetic reserve was established in Upper Twin Lake (Axolotl Lake) in the Gravelly Mountains in 1991. No natural reproduction occurs in Axolotl Lake

and the population is maintained by annually stocking age-1 fish obtained from onsite spawning of the Axolotl population. The purpose of the Axolotl Lake population was to establish an additional population with Big Hole River ancestry that could be used to augment natural grayling reproduction in the Big Hole or re-establish grayling in the Big Hole River in the event of extirpation. Direct introductions of captive fish are not recommended into the Big Hole River unless the population begins to rapidly lose genetic variation and is under immediate threat of extinction (Leary 1991). Axolotl Lake was the founding source of the reintroduced Ruby River population and is currently used for reintroduction efforts in the Madison River drainage.

The genetic diversity of the Axolotl Lake genetic reserve population diverged from the wild Big Hole River population following initial spawning efforts in Axolotl Lake (Leary 1991; Kovach et al. 2019). The allelic richness and expected heterozygosity of Axolotl Lake were found to be 8.5% and 1.0% lower, respectively, than the Big Hole River population in 2019. Genetic diversity was even lower when compared to the genetic diversity of the Big Hole River in the early 1990s when the population was founded (Kovach et al. 2019). Although it was recommended that direct introductions to the Big Hole River do not occur except when necessary, remote site incubators have been used to introduce eggs from the genetic reserve to the upper mainstem Big Hole River and its tributaries (e.g., Rock Creek, Wise River, Governor Creek, McVey Creek). Successful introductions, such as McVey Creek may have contributed to a reduction in the genetic variation of the wild population (Table 15; Kovach et al. 2019).

The conservation strategy for the Axolotl Lake brood is to maintain genetic diversity similar to the wild Big Hole River population through periodic genetic infusions, removal of fish which contribute to subsequent generations of the brood, and selective spawning of fish from different yearclasses. Successful implementation of this strategy will address all three management goals for UMR grayling.

Table 14. Conservation measures for the perpetuation of the Big Hole River Arctic grayling genetic reserve in Axolotl Lake.

Waterbody	Conservation Measure	Lead Agency	Cost
Axolotl Lake	Incubate eggs separately	FWP	\$5,000 operations & 6 existing staff
Axolotl Lake	Ensure equal contribution	FWP	\$5,000 operations & 6 existing staff
Axolotl Lake	Spawn different year classes	FWP	\$5,000 operations & 6 existing staff
Axolotl Lake	Equalize spawning contributions	FWP	\$5,000 operations & 6 existing staff
Axolotl Lake	Remove all parents which contribute to annual brood replacement	FWP	\$5,000 operations & 6 existing staff
Axolotl Lake	Infuse wild genes every ten years	FWP	\$5,000 operations & 6 existing staff

Progeny from the 2020 wild Big Hole River spawn were infused into the Axolotl genetic reserve beginning in 2022 when mature age-2 progeny were crossed with Axolotl fish. Continued wild infusion (as directed above) with the genetic reserve will reduce genetic divergence with the wild Big Hole River population. Axolotl Lake grayling will be the source for introductions into recently restored Big Hole tributaries (e.g., French Creek) and may also be used to augment the Big Hole River population if N_e declines below 50 for more than a generation (i.e., the population begins losing genetic variation, is potentially experiencing inbreeding depression, and is at risk of extinction).

Green Hollow Reservoir: A second genetic reserve was established in Green Hollow Reservoir in the Gallatin River drainage using fish from Axolotl Lake in 1998. Founder effects resulted in further genetic divergence from the wild Big Hole River population (Table 15; Kovach et al. 2019). The purpose of this population is to provide a source of UMR grayling for introductions outside of the Big Hole River drainage. Green Hollow Reservoir annually provides up to 400,000 eggs for reintroduction efforts in the Madison and Gallatin drainages.

Table 15. Expected heterozygosity (H_e) and allelic richness (A_r) of the Big Hole River and genetic reserve populations.

Sample	H_e	A_r
Big Hole 1980s	0.89	17.43
Big Hole 1990s	0.88	16.44
Big Hole 2000s	0.88	16.47
Big Hole early 2010s	0.89	16.41
Big Hole late 2010s	0.88	15.93
Green Hollow Reservoir	0.87	13.50
Axolotl Lake	0.87	14.58

The conservation strategy for the Green Hollow brood is to maintain a population large enough to provide adequate numbers of adult grayling to meet egg demands for reintroductions projects outside of the Big Hole River (Goals 2 and 3).

Handkerchief Lake: A genetic reserve for the Centennial Valley grayling population does not exist. Unsuccessful reintroductions of Arctic grayling into historically occupied waters of the Centennial Valley have been attempted using the progeny of wild fish from Red Rock Creek. Until recently, this population was considered resilient enough to withstand gamete removals for reintroductions throughout the valley. However, gamete removal is no longer possible given the recent decline in abundance of the grayling population in Upper Red Rock Lake which further necessitates the creation of a genetic reserve for the Centennial Valley grayling population. The purposes of this brood are:

1. Replicate genetic variation of Centennial Valley grayling to protect against extirpation
2. Minimize genetic differentiation between genetic reserve and wild Centennial Valley populations

Handkerchief Lake, in northwest Montana, is outside the native range of grayling but contained a viable population of introduced grayling for over 50 years until it was treated with piscicide in 2013 to remove hybridized cutthroat trout (Grisak and Marotz 2002). As part of the public scoping process, it was agreed that grayling would be reintroduced in Handkerchief Lake if the population had high conservation value. Following the fish removal project, attempts to replicate the Centennial Valley population were made in Handkerchief Lake using progeny from wild Red Rock Creek fish. However, those efforts have only included stocking 12,000 grayling fry and fingerlings whereas the original Handkerchief Lake grayling fishery was established with 711,000 fish stocked on 14 occasions during the 1950s and 1960s (Grisak and Marotz 2002). Few grayling are believed to occupy Handkerchief Lake based on targeted sampling and angler reports. Because of the low number of contributing parents, surviving fish have reduced genetic variation, which is not representative of the current or historical Centennial Valley population.

The conservation strategy for the Centennial Valley brood is to establish a genetically diverse grayling population of Centennial ancestry with population sizes sufficient to provide gametes for restoration projects in the Centennial Valley and Red Rock River sub-basin. FWP will establish a genetic reserve in Handkerchief Lake that most accurately represents the historical genetic variation of grayling in the Centennial Valley at abundances sufficient to serve as a brood source for conservation projects by introducing fish from multiple mountain lakes that appear to be primarily founded from the Centennial Valley grayling (i.e., Park, Bobcat, Schwinegar, Odell, Elizabeth lakes; Kovach et al. 2021). Once established, Handkerchief Lake will serve as the primary donor source for reintroductions into suitable habitat in the Centennial Valley and Red Rock River drainages. The following introduction strategies will be used to maximize success of genetic and demographic management goals (Table 16). Successful implementation of this strategy will ensure achievement of all three management goals for UMR grayling.

Table 16. Conservation strategies for the creation and perpetuation of a Centennial Valley Arctic grayling genetic reserve in Handkerchief Lake.

Waterbody	Conservation Measure	Lead Agency	Cost
Handkerchief Lake	Introduce remaining Centennial-origin fish in captivity	FWP	\$5,000 operations & 6 existing staff
Handkerchief Lake	Introduce grayling from at least three mountain lakes with primarily Centennial Valley ancestry into Handkerchief Lake	FWP	\$5,000 operations & 6 existing staff
Handkerchief Lake	Spawn at least 50 pairs from each of the three lakes using a 2x2 cross	FWP	\$5,000 operations & 6 existing staff
Handkerchief Lake	Use a combination of stocking fry and remote site incubators for introductions	FWP	\$5,000 operations & 6 existing staff
Handkerchief Lake	Collect genetic samples from all contributing parents for parentage-based tagging	FWP	\$5,000 operations & 6 existing staff
Handkerchief Lake	Annually collect 100 genetic samples from grayling in Handkerchief Lake for at least two generations following augmentation	FWP	
Handkerchief Lake	Infuse wild genes when demographically possible	FWP	\$5,000 operations & 6 existing staff

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